The separation of the nucleotides of an alkaline hydrolysate of ribonucleic acid by thin-layer chromatography

A number of purines, pyrimidines, nucleosides and nucleotides can be separated by thin-layer chromatography[1-3]. Using the procedure described below, guanylic, uridylic, adenylic and cytidylic acids released by alkaline hydrolysis of ribonucleic acid may be separated by this technique. Ribonucleotides from both yeast ribonucleic acid (L. Light & Co.) and from samples of nucleic acid extracted from rye seedlings by a modification of the method of TRIM[4] have been separated. Ribonucleic acid samples were hydrolysed in 0.3 *N* KOH at 37° for 18 h. Potassium was removed either by precipitation as the perchlorate or by passing the hydrolysate through a small column of cation exchange resin in the hydrogen form. The separated components of hydrolysates were identified by their ultraviolet absorption spectra and by the fact that each corresponded in position on the chromatograms to the appropriate pure nucleotide (British Drug Houses Ltd.) chromatographed by itself adjacent to the mixture.

The thin layers are prepared by suspending 0.5 g of a DEAE-cellulose powder suitable for thin-layer chromatography (Serva-Entwicklungslabor, Heidelberg, Germany) in 4 ml of water. The resulting slurry is poured onto an 18 × 6 cm glass plate and spread in a uniform layer along the plate using a glass rod. The rod is kept at a set distance above the plate by two pieces of wire (310 μ diam.) wrapped around the rod 5.5 cm apart, the diameter of the wire determining how thick a layer of the cellulose is spread. The layer is dried at room temperature and solutions of nucleotides applied about 2 cm from one end.

Two developments of the chromatograms are necessary, the first of which is by propan-1-ol–ammonia (sp. gr. 0.880-ANALAR)–water (60:30:10) at 40° allowing the front to reach at least 7 cm beyond the point of application of the nucleotides (45 min). After drying, the plate is developed again in the same direction by 0.24 *M* acetic acid at 20 to 25° until the front of this solvent just reaches the position reached by the front of the first solvent (15 min). The nucleotides are located by examination of the chromatogram in U.V. light.

The distances travelled by the individual nucleotides relative to the distance from the point of application to the position reached by the solvent fronts are: guanylic acid 0.21; uridylic acid 0.34; cytidylic acid 0.82; (the -2'- and -3'- phosphates of each of these are not resolved) and adenylic acid 0.53, 0.62 (the -2'- and -3'- phosphates separate). The use of a dilute weak acid (0.16 or 0.24 *M* acetic acid) in a single development did not separate guanylic from uridylic acid but did separate the two adenylic acid isomers and the cytidylic acid from one another and from the mixture of guanylic and uridylic acids. In the double development the first solvent separates guanylic acid (nearest the origin) from the other nucleotides and thus makes possible the complete separation on subsequent development with 0.24 *M* acetic acid. Satisfactory separations could not be achieved if the first solvent was freshly prepared using an ordinary reagent grade of 0.880 ammonia. Solvent so prepared had to be left to stand overnight before giving satisfactory results. Using ANALAR ammonia (British Drug Houses Ltd.,) a freshly prepared solvent gave a good separation.

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Department of Plant Physiology,
Imperial College of Science and Technology,
London (Great Britain)

T. A. DYER

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Separation of sulphidimines of sulphides on paraffin-treated paper

Several methods have been described for the paper chromatographic separation of the sulphidimines prepared from aliphatic sulphides and Chloramine T. LEAVER AND CHALLENGER[1] used both conventional techniques and reversed phase chromatography. PETRANEK AND VECERA[2] also used reversed phase techniques and the sulphidimine prepared by reacting the monochloroamide of *p*-nitrobenzenesulphonic acid with the sulphide instead of Chloramine T.

In studies on the metabolism of marine algae the volatile compounds produced in the cultures were examined by aspirating, into mercuric cyanide, mercuric chloride and mercuric acetate[3,4]. The volatile sulphides were precipitated as co-ordination compounds with mercuric chloride and converted to the sulphidimine by the method of LEAVER AND CHALLENGER[1]. The separation between the sulphidimines from dimethyl sulphide, ethyl methyl sulphide and diethyl sulphide was not sufficient to permit the distinguishing of sulphidimine-like spots, believed to be due to cyclic sulphides, from the former. Tetrahydrothiophene was found to behave in a similar manner when reacted with Chloramine T[5] and recrystallised from benzene.

The purpose of this communication is to suggest that the method of ASATOOR[6] using paraffin-impregnated paper, may with small modifications be used to give improved separation of the short-chained aliphatic sulphides chromatographed as sulphidimines.

Experimental

A strip of Whatman 3 MM paper (23 × 57 cm) was placed in a shallow tray (24 × 60 cm) containing a 5 % solution of liquid paraffin (sp. gr. 0.83–0.87) in 80–100° petroleum ether. The solution was allowed to flow over both surfaces of the paper by tilting the tray from side to side and by turning the paper. The paper was dried by